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Contract No. DE-AC36-08GO28308

Technical Report
NREL/TP-5100-82591
PNNL-SA-32722
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Suggested Citation
Swita, Marie, Teresa Lemmon, Ruoshui Ma, Joshua Taylor, Asanga B. Padmaperuma, Mariefel V. Olarte, Earl D. Christensen, and Jack R. Ferrell III. 2022. *Determination of Phenolic Groups in Bio-Oils Using Revised Folin-Ciocalteu Methods: Single Cuvette and Plate Reader. Laboratory Analytical Procedure (LAP), Issue Date: May 1, 2022.* Golden, CO: National Renewable Energy Laboratory. NREL/TP-5100-82591. PNNL-SA-32722. https://www.nrel.gov/docs/fy22osti/82591.pdf.

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This work was authored in part by the National Renewable Energy Laboratory, operated by Alliance for Sustainable Energy, LLC, for the U.S. Department of Energy (DOE) under Contract No. DE-AC36-08GO28308. Funding provided by U.S. Department of Energy Office of Energy Efficiency and Renewable Energy Bioenergy Technologies Office. The views expressed herein do not necessarily represent the views of the DOE or the U.S. Government.

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1. Introduction

1.1 Phenol components are ubiquitous in wood-derived bio-oils and biocrudes. Their reaction with other functional groups (e.g., aldehydes) may contribute to the formation of carbonaceous species and the expected thermal instability of pyrolysis oils. During hydrotreating, phenols can be recalcitrant species, requiring higher reaction temperatures than other oxygen-containing functional groups. Phenols are also present in upgraded products and have been shown to lead to catalyst deactivation during hydrotreating. They also are the first oxygenated functional group to reappear in the upgraded product, signaling catalyst deactivation.

1.2 The Folin-Ciocalteu (FC) method has been used to quantify phenolic groups in bio-oils and lignin-derived compounds. Two protocols exist: one with a single reagent and the other one with sodium carbonate (Na_2CO_3) as a second reactant to stabilize the color development [1,2]. We present a modified form of the dual-reagent system in this procedure.

1.3 This laboratory analytical procedure covers the determination of phenolic compounds in fast pyrolysis oils. It includes two methods, the first allowing for shorter analysis time at increased reaction temperature, and the second employing a longer analysis time but at room temperature. Additionally, the use of both a single cuvette and a plate reader are also presented.

2. Scope

2.1 This procedure has been optimized for the quantification of phenols in fast pyrolysis bio-oil.

2.2 Gallic acid has been used as a standard for the FC method due to its early application for measuring phenolics in winemaking [3]. Several bio-oil/biocrude relevant compounds were tested such as catechol, dimethoxyphenol, and guaiacol. This laboratory analytical procedure uses guaiacol as the standard.

2.3 This procedure only considers reacted samples with ultraviolet (UV) absorbance at 765 nm to be within 0.2–0.9 absorbance units. If the sample absorbance is not within this range, proper dilution or increasing the starting amount of the sample is needed.

3. Terminology

3.1 *Bio-oil* – The crude liquid product of converting lignocellulosic biomass into a liquid via fast pyrolysis or other thermochemical conversion process.

3.2 *Pyrolysis* – Chemical decomposition of organic materials by heating in the absence of oxygen.

3.3 *Fast pyrolysis* – Pyrolysis conducted with rapid heating and short residence time; typically less than 10 seconds.

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3.4 *Catalytic fast pyrolysis* – Fast pyrolysis conducted in the presence of a catalyst (can be either *in situ* or *ex situ*).

3.5 *Phenolics* – A family of compounds that contain at least one hydroxyl moiety connected to an aromatic ring.

3.6 *Folin-Ciocalteu reagent* – A mixture of phosphomolybdate and phosphotungstate acid complexes that forms chromogens with phenols and other reducing compounds.

4. Interferences

4.1 Sugars have been reported to cause interference [4]. Here, sugars were tested for interference at concentrations expected in these bio-oils. It was found that interference from sugars is negligible.

4.2 Quantification is based on the standard compound used. Guaiacol was found to have a similar response as gallic acid, which is traditionally used in the wine industry.

5. Apparatus

5.1 Analytical balance, accurate to 0.1 mg.

5.2 UV instrument, range 200–900 nm (e.g., Spectramax M-5).

5.3 Heating plate (optional).

6. Reagents and Materials Needed

6.1 Reagents

6.1.1 Folin-Ciocalteu reagent, reagent/analytical grade.

6.1.2 20% sodium carbonate, aqueous solution.

6.1.3 Deionized (DI) water.

6.1.4 Acetone (ACS reagent grade or better).

6.1.5 Guaiacol (FG, natural).

6.2 Materials

6.2.1 Scintillation vials (20 mL).

6.2.2 96-well microtiter plate, such as Costar 96-well cell culture cluster, or cuvettes (transparent between 300–900 nm).

6.2.3 Micropipettes.

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7. Environmental Safety and Health Considerations and Hazards

7.1 The Folin-Ciocalteu reagent is corrosive. Care should be taken in handling it.

7.2 Acetone is flammable.

7.3 Follow all applicable chemical handling procedures.

8. Sampling, Test Specimens, and Test Units

8.1 Bio-oil should be allowed to reach room temperature and be thoroughly homogenized to obtain a representative sample.

8.2 Sample absorbance should be between 0.2–0.9 absorbance units. If absorbance is lower than the lower limit, a more concentrated Solution B needs to be prepared (i.e., higher Stock 1:acetone ratio; see Section 9). If absorbance is higher than the limit, the initially prepared Solution B needs to be further diluted by acetone before the aliquot for FC analysis is taken.

8.3 The calibration curve of guaiacol with concentrations that span the absorbance of 0.1 to 1.5 absorbance units was found to have a second-order fit.

9. Analytical Procedure

9.1 Preparation of guaiacol standards:

9.1.1 Stock 1 (~1.5 wt %): Weigh 0.0750 g guaiacol into the vial. Tare vial with the cap on, remove cap, add 4.925 g of acetone (~6.25 mL), re-cap the vial, and record acetone mass.
Note: Acetone is volatile. It is recommended that the mass of acetone be taken with the vial capped to prevent solvent evaporation to obtain an accurate weight. Throughout the procedure, take care to prevent acetone evaporation.

9.1.2 Five levels of calibration standards gravimetrically (S1–S5) are prepared as follows:

9.1.2.1 S5 – Weigh 4.90 g of acetone into a vial. Add 0.50 g of Stock 1.

9.1.2.2 S4 – Weigh and mix 0.75 mL of S5 and 0.25 mL acetone.

9.1.2.3 S3 – Weigh and mix 0.5 mL of S5 and 0.5 mL acetone.

9.1.2.4 S2 – Weigh and mix 0.25 mL of S5 and 0.75 mL acetone.

9.1.2.5 S1 – Weigh and mix 0.1 mL of S5 and 0.9 mL acetone.

9.1.2.6 An aliquot of each standard level will be used in Section 9.3.

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9.2 Preparation of bio-oil sample solutions:

9.2.1 Sample stock (Solution A) – Weigh 0.3 g of bio-oil. Dilute in 10 mL of acetone. Record mass of acetone.

9.2.2 Sample stock dilution (Solution B) – Weigh 0.1 mL of Solution A and 0.9 mL of acetone. Record mass of acetone mix.

9.3 Folin-Ciocalteu reaction:

9.3.1 Preparation of standards:

9.3.1.1 Weigh 10.0 mL of water in a scintillation vial.

9.3.1.2 Add 200 microliters of FC reagent and 100 microliters of the calibration standard S1. Repeat 9.3.1.1 and 9.3.1.2 in separate scintillation vials for standards S2–S5 instead of calibration standard S1.
Note: Record weight after each liquid addition.

9.3.1.3 Proceed to 9.3.4.

9.3.2 Preparation of samples:

9.3.2.1 Weigh 10.0 mL of water in a scintillation vial.

9.3.2.2 Add 200 microliters of FC reagent and 60 microliters of bio-oil Solution B.
Note: Record weight after each liquid addition.

9.3.2.3 Proceed to 9.3.4.

9.3.3 Preparation of reaction blank:

9.3.3.1 Weigh 10.0 mL of water in a scintillation vial.

9.3.3.2 Add 200 microliters of FC reagent and 100 microliters of acetone.
Note: Record weight after each liquid addition.

9.3.3.3 Proceed to 9.3.4.

9.3.4 Mix the samples and let stand for 5 minutes.

9.3.5 Add 600 microliters of 20% aqueous sodium carbonate solution. Record the weight.

9.3.6 For shorter reaction time, heat the samples in a dry bath to 45°C for 30 min. Allow sample to cool down to room temperature before analysis if sample

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was heated. Otherwise, let the solution stand at room temperature for 2 hours.

9.3.7 Transfer appropriate amounts of the prepared FC-reacted solutions into separate cuvettes or approximately 200 microliters into three individual wells in a well plate. A minimum of three absorbance readings will be averaged during data analysis.
Note: The reaction blank will be subtracted from absorbances to obtain a blank corrected absorbance. If not using a well plate, use the blank for the reference cuvette.

9.3.8 Set the wavelength of the UV instrument to 765 nm.

9.3.9 Measure the absorbance of each of the samples following the instrument's control software. Report the average of at least three readings per sample.

10. Calculations

10.1 Calculate the Solution B dilution factor (SBDF):

10.1.1 $DF1 = \frac{total\ weight\ of\ Solution\ A}{weight\ of\ analyte}$

10.1.2 $DF2 = \frac{total\ weight\ of\ Solution\ B}{weight\ of\ Solution\ A\ aliquot}$

10.1.3 $SBDF = DF1\ x\ DF2$

10.2 Calculate FC dilution factor (FCDF):

10.2.1 $FCDF = \frac{total\ weight\ of\ the\ FC\ solution}{weight\ of\ Solution\ B\ aliquot}$

10.3 Calculate total dilution factor (TDF):

10.3.1 $TDF = SBDF\ x\ FCDF$

10.4 Calculate analyte weight fraction (AWF):

10.4.1 $AWF = \frac{weight\ of\ 100{-}microliter\ aliquot * TDF}{total\ weight\ of\ FC\ solution}$

10.5 Guaiacol calibration curve:

10.5.1 Plot the blank corrected absorbance vs. analyte weight fraction guaiacol of S1, S2, S3, S4, and S5.

10.5.2 Fit the curve using a second-order equation and use it to determine the concentration of the unknown.

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11. Report Format

11.1 Results can be summarized in a table or graph format for easy reference and/or comparison of values.

12. Precision and Bias

12.1 Intralaboratory precision showed less than 10% relative standard deviation (RSD) for fast pyrolysis oils.

12.2 The interlaboratory precision of this method has not yet been determined. The precision of this method will be updated once an interlaboratory study has been conducted.

13. Quality Control

13.1 Reported significant figures: Report results to one decimal place.

13.2 Replicates: Run all samples in at least duplicate preparation, with an average of three absorbance readings per individual sample.

14. References

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